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ORIGINAL PAPER

Patterns of laccase and peroxidases in coarse woody debris of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris* and their relation to different wood parameters

Tobias Arnstadt[1] · Björn Hoppe[2,3] · Tiemo Kahl[3] · Harald Kellner[1] · Dirk Krüger[2] · Claus Bässler[4] · Jürgen Bauhus[3] · Martin Hofrichter[1]

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Abstract Lignin and its degradation, particularly in forest ecosystems, play a major role in the global carbon cycle. Filamentous fungi equipped with extracellular oxidoreductases (oxidative enzymes), i.e., laccase, manganese-dependent peroxidases and several other peroxidases, are the key players in the bioconversion of lignin. In particular, for coarse woody debris (CWD), this process is poorly understood and the activities of laccase and peroxidases have never been studied on a large field scale. We investigated the activities of these enzymes in 701 samples of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris* CWD across three regions in Germany and analyzed their dependence on pH, water content, wood density, total lignin, organic extractives, metals, water-soluble lignin fragments and fungal species richness. Respective enzyme activities were present in 79 % of all samples, and the activities were highly variable and more frequent in *F. sylvatica* than in coniferous wood. Logistic regressions and correlations between enzyme activities and the variables revealed that the fungal community structure and the amount of water-soluble lignin fragments are most important determinants, and that the prevalent acidic pH in CWD is suitable to facilitate laccase and manganese peroxidase activities. Concentrations of metals (manganese, copper, iron) were sufficient to ensure synthesis and functioning of relevant enzymes. Based on this large field study, we conclude that laccase and peroxidases in CWD are highly relevant for lignin degradation, but the variable pattern of their secretion is the result of a complex array of wood parameters and the fungal community structure, which could only partly be resolved.

Keywords Laccase · Manganese peroxidase · General peroxidase · Dead wood · Lignin · Wood rot

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✉ Tobias Arnstadt
tobias.arnstadt@tu-dresden.de; arnstadt@ut-arnstadt.de

[1] Department of Bio- and Environmental Sciences, International Institute Zittau, Technische Universität Dresden, Zittau, Germany

[2] Department of Soil Ecology, UFZ - Helmholtz Centre for Environmental Research, Halle (Saale), Germany

[3] Chair of Silviculture, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg im Breisgau, Germany

[4] Bavarian Forest National Park, Freyunger Str. 2, 94481 Grafenau, Germany

Introduction

The degradation of lignin is of fundamental importance for the global carbon cycle (Dixon et al. 1994; Lundell et al. 2014; Weedon et al. 2009), especially in dead wood that represents globally 5 % of organic carbon in forest ecosystems (FAO 2010). Lignin represents a barrier in wood that protects cellulose and hemicelluloses from microbial degradation (Kirk 1984). Only specialized filamentous fungi of the phylum Basidiomycota and some Ascomycota can substantially degrade lignin (Dix and Webster 1994; Liers et al. 2006). Due to the high lignin content in wood (20–40 %; Fengel and Wegener 2003), this process is essential for the mineralization of dead wood and increases the accessibility of the carbohydrates for other organisms such as non-ligninolytic fungi, bacteria or arthropods (Stokland et al. 2012).

Ecophysiologically, saprotrophic wood-inhabiting fungi can be classified, as a result of different degradation strategies, by the type of rot they are causing: white rot, brown rot and soft rot (type I and II) (Hatakka and Hammel 2011). All of these fungi are able to utilize and mineralize cellulose and hemicelluloses by different hydrolytic enzymes. The types of rot differ mainly in the ability and strategy to overcome the lignin barrier as well as in the time shift between lignin and polysaccharide degradation (Hatakka and Hammel 2011; Schwarze 2007). Typical white-rot fungi (WRF) actively secrete a set of extracellular oxidative enzymes (oxidoreductases EC 1.xx.x.x), i.e., peroxidases (EC 1.11.1.x) and phenol oxidases of the laccase type (Lacc, EC 1.10.3.2) (Lundell et al. 2010). Fungal secretory peroxidases can be phylogenetically divided into three large groups: (1) high-redox potential class II peroxidases, i.e., manganese peroxidase (MnP, EC 1.11.1.13), lignin peroxidase (LiP, EC 1.11.1.14), versatile peroxidase (VP, EC 1.11.1.16) and low-redox potential generic peroxidase (GP, EC 1.11.1.7), (2) dye-decolorizing peroxidases (DyP, EC 1.11.1.19) and (3) unspecific peroxygenases (UPO, EC 1.11.2.1) (Hofrichter 2002; Hofrichter and Ullrich 2014; Hofrichter et al. 2010). Among these peroxidases, only MnP, LiP and VP were shown to disintegrate polymeric lignin, while DyP and UPO have merely been demonstrated to cleave non-phenolic β-O-4-lignin model dimers (Hofrichter et al. 2010), and GP-type enzymes, on contrary, are rather involved in the polymerization of lignin (Kjalke et al. 1992). Equipped with varying sets of these oxidative enzymes, WRF reduce the lignin content of dead wood remarkably (Schwarze 2007).

In contrast, brown-rot fungi (BRF) do not secrete peroxidases, but they are applying a special radical mechanism with hydroxyl radicals (HO·) as oxidant, produced by the non-enzymatic Fenton reaction. Thus, BRF are not able to mineralize lignin to carbon dioxide (CO_2) as WRF do, and lignin is only modified, while the polymeric structure is kept intact (Hatakka and Hammel 2011). But BRF can efficiently attack hemicelluloses and cellulose, which leads to a relative increase in lignin frequently found in soft wood, since BRF prefer conifers (Hatakka and Hammel 2011; Ryvarden and Gilbertson 1993; Schwarze 2007).

Among the soft-rot fungi (SRF), Lacc is the most frequently found oxidative enzyme. These fungi (often filamentous ascomycetes, e.g., of the order Xylariales) mineralize lignin only to some extent, while cellulose and hemicelluloses are substantially degraded (Hatakka and Hammel 2011; Liers et al. 2006; Stokland et al. 2012).

Although lignin is widespread across different ecosystems, fungal degradation of wood and the involved enzymes has mainly been studied under artificial conditions in the laboratory. Experiments with pure fungal cultures on sterilized wood blocks and chips showed that each fungus provokes a unique temporal pattern of Lacc and peroxidase activities and that each of these enzymes contribute to a specific molecular mass distribution of water-soluble lignin fragments during degradation (Fackler et al. 2006; Hofrichter et al. 2001; Liers et al. 2011). Other laboratory experiments revealed the dependence of laccase and peroxidase production and their catalytic functioning on pH (e.g., acidic optimum of Lacc and MnP; Baldrian 2006; Hofrichter et al. 2010; Kirk and Cullen 1998) and specific metals. Thus, copper (Cu^{2+}) is part of the catalytic center of Lacc and induces its production (Baldrian 2006; Levin et al. 2005), iron (Fe^{3+}) is the central ion of protoporphyrin IX (heme) in the active site of most peroxidases including MnP, LiP and VP (Hofrichter et al. 2010), and manganese (Mn^{2+}) stimulates the production of MnP and is essential for the catalytic cycle of this enzyme (Brown et al. 1990; Hofrichter 2002).

In contrast to these detailed studies in the laboratory, the degradation of dead wood and the role of Lacc and peroxidases in the field have only scarcely been investigated with the exception of three recent studies. Valaskova et al. (2009) reported mean activities of Lacc and MnP from seven wood samples of different tree species, and van der Wal et al. (2015) mentioned activities of Lacc in 46 tree stumps of *Quercus robur*. Větrovský et al. (2010) determined Lacc and MnP activities in *F. sylvatica* and *B. pendula* samples (15 each) and found a significant level of spatial heterogeneity of these enzyme activities in decaying wood. However, our understanding of the factors that contribute to the considerable variability of Lacc and peroxidase activities in CDW is still limited.

In other field studies regarding dead wood decomposition, the actual decay activity of the whole decomposing community in CWD was assessed by respiration measurements, mass loss over time, decomposition rates, changes in the wood density or by the lignin content often along with molecular data (Boddy et al. 1989; Fukasawa et al. 2009a; Herrmann and Bauhus 2013; Rajala et al. 2012). However, to our best knowledge, no study has dealt with oxidative enzyme activities in CWD using a high sample number at a large spatial scale or analyzed their dependence on diverse predictors.

Therefore, we analyzed here Lacc and peroxidase activities in naturally occurring CWD of *F. sylvatica*, *P. abies* and *P. sylvestris* in temperate forest ecosystems within three regions across Germany. Based on laboratory studies, we hypothesize (H1) that these enzyme activities in CWD are promoted by acidic pH and positively correlated with the concentrations of Mn, Fe and Cu as well as with water-soluble lignin fragments. Moreover, we expect a positive correlation of Lacc and peroxidase activities with the water content of CWD and a negative correlation with wood density, since similar results were reported for respirations rates, which are partly the result of lignin degradation (Hagemann et al. 2010; Herrmann and Bauhus 2013; Hofrichter et al. 1999). Fukasawa et al. (2009a) and Rajala et al. (2012) demonstrated a distinct fungal community development during deadwood degradation, and the composition of the fungal community was found to be the best predictor for mass loss in older stumps of *Q. robur* (van der Wal et al. 2015). In this context, we hypothesize (H2) that these fungal community changes will trigger the activities of Lacc and peroxidases. Finally, we assume a direct response of enzyme activities to the content of total lignin.

Materials and methods

Study site

This study was conducted in the context of the Biodiversity Exploratories (www.biodiversity-exploratories.de), (Fischer et al. 2010), a long-term and large-scale experiment for functional biodiversity research in Germany. The studied forests are located in the following three regions: (1) UNESCO Biosphere Reserve Schorfheide-Chorin (3–140 m a.s.l.) in the glacially formed lowland in north-eastern part of Germany, (2) National Park Hainich-Dün and its surrounding areas (285–550 m a.s.l.) in the hilly lands of central Germany and (3) UNESCO Biosphere Reserve Schwäbische Alb and adjacent areas (460–860 m a.s.l.) in the low mountain range of southwestern Germany. The annual mean temperatures are in the range of 8–8.5, 6.5–8 and 6–7 °C, and the annual mean precipitation varies between 500–600, 500–800 and 700–1000 mm for Schorfheide-Chorin, Hainich-Dün and Swabian Alb, respectively (Fischer et al. 2010).

Thirty plots (each 100 × 100 m, nine in Schorfheide-Chorin, nine in Schwäbische Alb, 12 in Hainich-Dün) of different forest management types were investigated. More detailed information about all forests plots is given in Fischer et al. (2010), Hessenmöller et al. (2011) and Luyssaert et al. (2011).

Sample collection

In April 2009, autochthonous, downed CWD was sampled within the plots. To cover a great spectrum of CWD, on each plot, slightly, moderately and strongly decayed logs were selected in the diameter classes 7–20 cm and >20 cm for *F. sylvatica* and the dominant coniferous tree species (*P. abies* or *P. sylvestris*). Due to limited availability of naturally occurring logs, not all diameter classes and decay stages could be covered for each species per plot. In total, 190 logs were selected in the three explanatories (Table 1), of which 30 % were slightly, 40 % moderately and 30 % strongly decayed (Online Resource: Table S1). The size classes were nearly equally represented for *F. sylvatica* and *P. abies*. Only for *P. sylvestris*, the size class 7–20 cm was overrepresented with 78 % (Online Resource: Table S2).

From May to June 2009, dead wood samples were collected in the form of chips by drilling samples from the selected logs. A cordless Makita BDF451 (Makita, Anjo, Japan) equipped with a 42-cm-long wood auger with 2 cm diameter was used. From each log less than five meter long, three samples were taken. From logs longer than five meter, a further sample for every additional 5 meter was taken. The sampling positions were equally spaced over the whole length of the log, and the drill was introduced at an angle around 45° to a vertical line perpendicular to the long axis. Depending on the log diameter at the sampling point, the auger was either drilled trough or inserted into its maximum length. Between the different samplings, the auger was flamed and wiped with ethanol to sterilize it. To avoid overheating, the drill operated slowly and the procedure was paused periodically. If drilling was not possible, due to fragile wood, a representative piece of wood was cut using a handsaw. In total, 399, 210 and 92 samples were taken from *F. sylvatica*, *P. abies* and *P. sylvestris*, respectively. The samples were kept on dry ice and later weighted and divided into three subsamples: (1) stored at −18 °C for enzymatic studies, (2) stored at −80 °C for F-ARISAs and (3) directly dried at 65 °C until constant mass for determination of the density and water content (Hoppe et al. 2014).

Sample preparation

Frozen samples (−18 °C) were ground by an ultra-centrifugal mill (<1 min, 10,000 rpm, ZM 1000, Retsch GmbH, Haan, Germany) with a fixed ring sieve (1 mm mesh) and cooled by dry ice and liquid nitrogen.

Afterward, the samples were extracted with distilled water in a round-bottomed flask by vigorous shaking on a rotary shaker at 120 rpm for 2 h at a temperature of 11 °C. The volume of distilled water was adjusted to the actual dry mass of samples using a ratio of 10 ml for 1 g. Extracts were filtered through a glass fiber filter (VWR, particle retention: 1.6 µm). Aqueous extracts were centrifuged (10 °C, 10 min, 16,100 × *g*), and the supernatants analyzed for activities of Lacc and peroxidases, pH, bioavailable metals and the molecular mass distribution of water-soluble lignin fragments. Retained extracted wood samples were dried at 60 °C to a constant mass and used to determine organic extractives and Klason lignin.

Table 1 Parameters of studied coarse woody debris

	Fagus sylvatica	*Picea abies*	*Pinus sylvestris*
Number of logs	112	55	23
In Schorfheide-Chorin	28	0	23
In Hainich-Dün	46	19	0
In Schwäbische Alb	38	36	0
Number of samples	399	210	92
Log diameter (cm)[a]	26 ± 19 (6–90)	24 ± 12 (10–70)	19 ± 10 (10–51)
Log length (m)[a]	8 ± 6 (2–33)	9 ± 6 (2–31)	10 ± 6 (3–24)

[a] Mean values ± standard deviation, and in brackets, the ranges (min and max) are given

Activities of Lacc and peroxidases

Activities were photometrically measured in the aqueous extracts in 96-well plates (F-bottom, Greiner Bio-One GmbH, Frickenhausen, Germany) with a plate reader (Infinite M200, Tecan, Männedorf, Switzerland) by following the oxidation of ABTS (2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid), $\varepsilon_{420} = 36.0\ cm^{-1}\ mM^{-1}$). After mixing 50 µl sample extract and 195 µl ABTS buffer solution, the assay contained 0.3 mM ABTS and 50 mM sodium malonate buffer (pH 4.5) (Hahn et al. 2013; Liers et al. 2011). At first, Lacc activity was measured over 5 min immediately after addition of the sample extract under two different conditions in order to differentiate the subsequent peroxidase assays: (1) in sodium malonate buffer additionally containing ethylenediaminetetraacetic acid (EDTA, Titriplex III, 0.5 mM) for complexing naturally occurring free metal ions (in first place Mn^{2+}; Větrovský et al. 2010) and (2) in sodium malonate buffer with Mn^{2+} ($MnCl_2$, 0.5 mM) and without EDTA. General peroxidase (GenP) activity was measured by following the increase in absorption at 420 nm after addition of hydrogen peroxide (H_2O_2, 0.1 mM) in the EDTA-containing assay solution for 5 min. GenP activity was corrected by subtraction of the determined Lacc activity. In parallel, manganese-dependent peroxidase (MnP) activity was measured in the assay solution with Mn^{2+} after addition of 0.1 mM H_2O_2 over 5 min. MnP activity was finally calculated by subtraction of Lacc (with Mn^{2+}) and GenP activities. The GenP activity stands for the sum activity of a genetically diverse set of fungal peroxidases that can include LiP, VP, GP, UPO and DyP in the absence of Mn^{2+}, which is why the GenP activity has also been described as activity of manganese-independent peroxidases (MiP; Kellner et al. 2014). All measurements were taken in duplicate and were repeated when the absorbance increase in the two measurements was strongly deviating. For further analyses, the mean values were used. All enzymatic activities are given in international units (U; $1\ U = 1000\ mU = 16.67 \times 10^{-9}$ kat) defined as the amount of enzyme that forms 1 µmol of product per minute under the assay conditions used; they were related to the original dry mass of wood: $mU\ g^{-1}$.

Physico-chemical properties of the wood samples

To assess water-soluble lignin fragments, high-performance size exclusion chromatography (HPSEC) was used to determine their molecular mass distribution in the aqueous extracts (Hofrichter et al. 2001; Liers et al. 2011). The HPLC system (Agilent 1100 Liquid Chromatography; Agilent Technologies, Santa Clara, USA) was equipped with a diode array detector and fitted with a HEMA-Bio linear column (8 × 300 mm, 10 µm) from Polymer Standard Service (Mainz, Germany). The mobile phase consisted of 20 % acetonitrile and 80 % of aqueous buffer (pH 10; 0.34 % (m/v) NaCl and 0.2 % (m/v) K_2HPO_4). The following separation parameters were used: flow rate 1 ml min^{-1}, detection wavelength 280 nm and injection volume 50 µl. Sodium polystyrene sulfonates (0.8–1010 kDa, Polymer Standard Service, Mainz, Germany) served as molecular mass standards. The amount of water-soluble lignin fragments was derived from the peak area under the elution profile calibrated with a chlorolignin standard (Lackner et al. 1991; Soares and Duran 2001).

Bioavailable metals (copper, manganese and iron) in CWD were determined in the aqueous extracts using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to manufacturers' specifications. The ICP-OES device Optima 3000 (PerkinElmer Inc., Waltham, MA, USA) was calibrated using the ICP multi-element standard solution IV (Mn, Fe, Cu; Merck, Darmstadt, Germany) from 0 to 10 mg l^{-1}. The ICP-MS Elan DRC-e (PerkinElmer) device was calibrated using ICP multi-element standard solution VI (Mn, Fe, Cu; Merck) in 2, 15, 50 and 500 µg l^{-1} (only Fe) concentrations.

The content of lignin and organic extractives of the solid wood samples was determined after aqueous extraction and drying (at 60 °C). Each sample was extracted by accelerated solvent extraction (Dionex ASE 200) with acetone (cell size: 5 ml; preheating: 1 min; static equilibration period: 100 °C, 1500 PSI, 3 × 5 min; flush: 80 %; purge: 300 s). From the resulting extract, acetone was evaporated at 60 °C under nitrogen flush. The remaining residues were dried at 120 °C over night and weighted. The content of organic extractives was expressed per gram of original dry mass of wood (Liers et al. 2011).

The extracted samples were ground by a planetary ball mill (5 min, 650 rpm, Pulverisette 7, Retsch, Idar-Oberstein, Germany) to a fine powder. Klason lignin content was measured gravimetrically as dry weight of solids after sequential hydrolysis with sulfuric acid (72 % (v/v) at 30 °C for 1 h and 2.4 % (v/v) at 120 °C for 1 h; Effland 1977). Acid-soluble lignin was measured photometrically according to Dence (1992). The UV absorbance of the hydrolysate containing acid-soluble lignin was recorded at 205 nm ($\varepsilon = 110\ g^{-1}\ cm^{-1}$). The Klason lignin and acid-soluble lignin contents were expressed per gram of original dry mass of wood.

Wood density was calculated from the dry weight (65 °C) divided by the sample volume (volume of the drilled hole). Water content is expressed as mass of water per unit of dry mass of wood.

Fungal community in the wood samples

The total community DNA from 1 g wood powder of each sample was isolated using a modified CTAB protocol (Doyle and Doyle 1987). Nine hundred microliters of CTAB was added to the sample, and the nucleic acids were separated from proteins and cell debris by adding 500 µl of 24:1 chloroform:isoamyl alkohol (Carl Roth, Karlsruhe, Germany) followed by another chloroform step (Carl Roth, Karlsruhe, Germany). DNA was precipitated by washing the sample twice with ethanol (Merck, Darmstadt, Germany). Dried pellets were eluted in 100 µl molecular water (AppliChem, Darmstadt, Germany).

F-ARISA PCR (fungal automated ribosomal intergenic spacer analysis PCR; Ranjard et al. 2001; Purahong et al. 2014) of each DNA extract was done in two replicates using a FAM-labeled primer ITS1-F (5′-CTTGGTCATT TAGAGGAAGTAA-3′, Gardes and Bruns 1993) and unlabeled ITS4 (5′-TCCTCCGCTTATTGATATGC-3′, White et al. 1990) in 30 µl reaction mixtures containing 6 µl FIREPol 5× Master Mix (Solis BioDyne, Tartu, Estonia), 15 µM of each Primer and 1 µl template DNA. PCR was performed with an initial denaturation step at 95 °C for 5 min followed by 35 cycles at 95 °C for 60 s, 55 °C for 60 s and 72 °C for 75 s. Elongation was completed with a final step at 72 °C for 7 min.

PCR products were purified using the E.Z.N.A.® Cycle-Pure Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). 10 ng of each purified PCR product was dissolved in 14 µl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA) with 0.1 µl of internal size standard Map Marker 1000 ROX (BioVentures, Inc., Murfreesboro, TN, USA). After denaturation for 5 min at 95 °C, samples were chilled on ice for at least 10 min. Length heterogeneity of fungal ITS fragments (approximately 450–1000 bp) was detected by capillary electrophoresis (ABI 3730xl, Applied Biosystems). Electrophoretic conditions were as follows: seven-second-injection at 1.6 kV and separation at 15 kV for 3800 s. Row profiles were analyzed using Gene Mapper software 4.0 (Applied Biosystems). All peaks above a threshold of 100 fluorescence units, which were present in both technical replicates, were considered for further analyses. OTU binning was performed with the interactive and automatic R binning script (Ramette 2009) using R (R Core Team 2014). According to the script and its correlation values, a window size of two was chosen.

Statistics

All statistical analyses were performed in R 3.0.3 (R Core Team 2014). The data were tested for normal distribution applying the Shapiro–Wilk normality test. Differences between tree species were tested by the Kruskal–Wallis test for significance ($p < 0.05$) that was followed by a paired Mann–Whitney test with Bonferroni correction.

Separately for each tree species, the influence of all physico-chemical properties, species richness and fungal community (independent variables) on the activity of Lacc, GenP and MnP was analyzed by fitting multiple linear and generalized linear mixed-effect models with region, plot and CWD log as nested random effects. At first, the fungal community matrix was arcsin transformed and ordinated with NMDS applying the function “metaMDS” in vegan package (2.0–10; Oksanen et al. 2013). The first two axes (Online Resource: Fig. S1) were extracted and used as proxy for the fungal community in the multiple models. Before modeling, the normal distribution of all independent variables was checked and, if necessary, transformed (Online Resource: Table S3). To avoid highly correlated independent variables in the models, the co-correlation of the transformed independent variables was checked by correlation analysis (Online Resource: Table S4-S6). Since water content and wood density were highly correlated as well as water-soluble lignin fragments, copper, iron and manganese showed high correlation, only the water content and water-soluble lignin fragments were used in the multiple models, and the others were excluded.

Since 21 % of all samples did not contain any of the analyzed enzyme activities ($<1\ mU\ g^{-1}$, Table 2), the influence of the independent variables on the probability to detect Lacc, GenP or MnP was analyzed by logistic regression. For this analysis, the enzyme data were converted into presence ($\geq 1\ mU\ g^{-1}$) or absence

Table 2 Percentage of CWD samples with different combinations of Lacc and peroxidases $\geq$1 mU g^{-1}

Lacc[a]	GenP[a]	MnP[a]	*F. sylvatica* (%)	*P. abies* (%)	*P. sylvestris* (%)
−	−	−	9	35	40
+	−	−	13	18	14
−	+	−	4	4	3
−	−	+	1	1	3
+	+	−	12	7	11
+	−	+	15	6	9
−	+	+	5	3	1
+	+	+	42	26	18
Samples containing Lacc			81	57	52
Samples containing GenP			63	40	34
Samples containing MnP			63	36	32
Samples containing any oxidoreductase			91	65	60

[a] Combinations are with (+) and without (−) the corresponding enzyme activities

(<1 mU g^{-1}) of the corresponding enzyme activities per analyzed sample. Logistic regressions were modeled as generalized linear mixed model using the Lme4 package (1.0–6; Bates et al. 2014) with region, plot and CWD log as nested random effects, and the independent variables were *z*-scaled. Before fitting the multiple logistic models, a univariate linear and quadratic logistic model was fitted for each enzyme type in dependence of each independent variable. They were plotted together with the mean probability and standard deviation from eight intervals across the range of the independent variable to evaluate graphically the need of a quadratic term according to Crawley (2012). Further significance ($p < 0.05$) of the quadratic term was tested by an analysis of variance (ANOVA) comparing the linear and quadratic model.

In a second step, we analyzed the dependence of present enzyme activities on the independent variables. To exclusively analyze samples where the enzyme type of interest is present, the data set was reduced to samples containing the corresponding enzyme with activities of $\geq$1 mU g^{-1}. The enzyme activities were checked for normal distribution and transformed to better fit to a normal distribution (Online Resource: Table S7). Before fitting the multiple linear models, the enzyme activities were plotted in dependence of the independent parameters to explore the relevance of quadratic terms. The multiple linear mixed-effects models were fitted with the function "lme" from the "nlme" package (Pinheiro et al. 2013) with region, plot and CWD log as nested random effects. Therefore, the enzyme activities and the independent parameters were *z*-scaled.

Best model selection was conducted for logistic regression and linear regressions by the *R* function "dredge" from the *R* package "MuMIn" (Bartoń 2015), an automated full information criteria-based model selection approach, evaluated on the basis of Bayesian information criteria (BIC). To estimate the explanatory power of the final models, "Nakagawa & Schielzeth's minor *r*-square" (Nakagawa and Schielzeth 2013) was calculated using the *R* function "*r*.squared" supplied by Lefcheck (2014).

Results

Enzyme activities in wood samples

One of the three types of oxidoreductase activities (Lacc, GenP and MnP) was found in 91, 65 and 60 % of all samples of *F. sylvatica*, *P. abies* and *P. sylvestris*, respectively. Combinations of these enzyme activities were observed in 42, 26 and 18 % of all the wood samples in regard to tree species (Table 2). Vice versa, no oxidoreductase activity was detected in 9, 35 and 40 % of *F. sylvatica*, *P. abies* and *P. sylvestris* samples, respectively. In general, a wide range of enzyme activities from 0 up to 1209, 333 and 929 mU g^{-1} were measured for Lacc, GenP and MnP, respectively (Table 3). All maximum enzyme activities were found in *F. sylvatica* CWD. The majority of samples showed enzyme activities lower than 10 mU g^{-1}. In general, a high variability of enzymatic activities was observed within individual logs. The mean of all coefficients of variation in each individual log ranged from 118 to 155 % for each enzyme and each tree species (Table 3).

Significant ($p < 0.01$) higher activities of Lacc, GenP and MnP were found in wood of *F. sylvatica* than in coniferous wood (Table 3). In all CWD tree species, peroxidases showed significantly ($p < 0.01$) lower activities than Lacc and were detected in a smaller percentage of samples (Table 2). However, the percentage of samples with GenP or MnP activities was similar. The activities of

Table 3 Summary statistics of measured Lacc and peroxidase activities

CWD type	Enzyme activity (mU g^{-1})							Mean SD in CWD logs (mU g^{-1})	Mean CV in CWD logs (%)
	Min	$Q_{25\ \%}$	Median	$Q_{75\ \%}$	$Q_{95\ \%}$	Max			
Laccase									
F. sylvatica	0	2	12	57	341	1209	a[a]	78	118 ± 44
P. abies	0	0	1	10	112	784	b	33	148 ± 42
P. sylvestris	0	0	1	5	62	287	b	15	129 ± 51
General peroxidase									
F. sylvatica	0	0	2	11	73	333	a	17	125 ± 46
P. abies	0	0	0	3	32	306	b	9	154 ± 52
P. sylvestris	0	0	0	1	8	24	b	2	140 ± 66
Manganese peroxidase									
F. sylvatica	0	0	2	16	102	929	a	30	131 ± 45
P. abies	0	0	0	2	51	375	b	14	155 ± 50
P. sylvestris	0	0	0	1	3	9	b	1	155 ± 56

The minimum, the 25 %, 50 % (median), 75 % and 95 % quantiles and maximum of the different enzyme activities in samples from *F. sylvatica*, *P. abies* and *P. sylvestris* are given. Standard deviation (SD) and coefficient of variation (CV) within each individual log were calculated and given as mean SD and CV over all logs

[a] Different letters (a,b) indicate statistically significant differences between tree species for the corresponding enzyme activities at $p < 0.05$

all these enzymes showed a strong positively skewed distribution.

Physico-chemical properties and fungal species richness of CWD samples

Most physico-chemical properties showed significant differences between *F. sylvatica* CWD and coniferous wood (Table 4). The pH values were in the range from 3.0 to 7.5 and had a significant lower median in coniferous wood (pH 4.4) than in *F. sylvatica* (pH 5.1).

The amount of water-soluble lignin fragments varied between 0.2 and 99.4 mg g^{-1} and showed a significant lower median in coniferous wood (6.8 mg g^{-1}) compared to wood of *F. sylvatica* (9 mg g^{-1}). The main fraction of water-soluble lignin fragments had a molecular mass around 1 kDa (Fig. 1). This fraction was found in 74, 65 and 69 % of all samples in *F. sylvatica*, *P. abies* and *P. sylvestris*, respectively. The second smaller peak representing a fraction around 40 kDa was present in 49, 10 and 11 % of all samples in *F. sylvatica*, *P. abies* and *P. sylvestris*, respectively.

Water-soluble (i.e., bioavailable) manganese, copper and iron had median concentrations of 14.2, 0.35 and 1.91 µg g^{-1}, respectively (Table 4), and showed only slight differences between the tree species. Among each other and with water-soluble lignin fragments, they showed strong correlations (Online Resource: Table S4-S6). The corresponding median metal concentrations in the aqueous phase of CDW (deduced from the water content of CDW) were 206, 4 and 30 µM for manganese, copper and iron, respectively (Online Resource: Table S8).

The median contents of total lignin and wood extractives in coniferous wood were significantly higher than in *F. sylvatica* (Table 4). CWD density increased from *P. abies* over *P. sylvestris* to *F. sylvatica* with a median of 0.24, 0.29 and 0.33 g cm^{-3}, respectively. In contrast, the water content did not show significant differences between the tree species ranging from 0.3 to 11.3 g g^{-1} (Table 4). Water content and wood density were found to be strongly negatively correlated (R: −0.82, −0.79 and −0.85 for *F. sylvatice*, *P. abies* and *P. sylvestris*, respectively).

The fungal species richness exhibited significantly higher OTU numbers in coniferous CWD than in *F. sylvatica* CWD with medians of 15, 19 and 21 OTUs for *F. sylvatica*, *P. abies* and *P. sylvestris*, respectively (Table 4).

Probability to detect Lacc, GenP and MnP activities

Due to the high percentage of samples that did not show any activity of Lacc, GenP or MnP (Table 2), we modeled in a first step the probability to detect respective enzymatic activities (≥1 mU g^{-1}) in dependence of the physico-chemical properties, the first and second axis of the NMDS ordination for the fungal community and species richness as logistic regressions.

The final models for the probability to detect Lacc, GenP and MnP activities in *F. sylvatica* CWD showed only low minor r-squares (0.03–0.11), whereas for the models of coniferous CWD, the values were clearly higher (0.14–0.52; Table 5). The variable that contributed most frequently (five times) to the final models was the

Table 4 Summary statistics of physico-chemical properties and species richness

	CWD type	Min	$Q_{25\ \%}$	Median	$Q_{75\ \%}$	Max	
pH							
	F. sylvatica	3.4	4.7	5.1	5.7	7.4	a[a]
	P. abies	3.0	4.0	4.4	4.9	7.5	b
	P. sylvestris	3.1	4.1	4.4	4.7	6.3	b
c_{Mn} (µg g^{-1})							
	F. sylvatica	0.1	5.4	13.0	31.1	361.3	a
	P. abies	0.3	7.7	13.1	34.3	193.9	a,b
	P. sylvestris	2.4	9.3	20.9	32.3	106.6	b
c_{Cu} (µg g^{-1})							
	F. sylvatica	0.1	0.3	0.4	0.5	3.0	a
	P. abies	0.0	0.2	0.3	0.5	2.2	b
	P. sylvestris	0.1	0.2	0.3	0.4	1.5	b
c_{Fe} (µg g^{-1})							
	F. sylvatica	0.1	1.1	1.9	3.3	44.8	a
	P. abies	0.1	0.9	2.0	4.7	38.6	a
	P. sylvestris	0.1	0.7	1.5	3.3	13.7	a
Amount of water-soluble lignin fragments (mg g^{-1})							
	F. sylvatica	0.9	5.1	9.0	14.7	99.4	a
	P. abies	0.2	3.4	6.8	11.5	48.4	b
	P. sylvestris	0.3	2.5	6.8	9.8	22.8	b
Extractives (%)							
	F. sylvatica	0.1	0.5	0.7	1.2	7.0	a
	P. abies	0.2	1.1	1.8	2.8	13.1	b
	P. sylvestris	0.8	2.6	4.0	9.1	24.1	c
Total lignin (%)							
	F. sylvatica	19	26	28	31	73	a
	P. abies	23	30	32	41	90	b
	P. sylvestris	19	29	31	37	59	b
Density (g cm^{-3})							
	F. sylvatica	0.03	0.19	0.32	0.48	0.69	a
	P. abies	0.02	0.16	0.24	0.33	0.48	b
	P. sylvestris	0.08	0.17	0.29	0.38	0.53	a
Water content (g g^{-1})							
	F. sylvatica	0.30	0.69	1.25	2.42	11.27	a
	P. abies	0.34	0.70	1.30	2.53	6.90	a
	P. sylvestris	0.30	0.70	1.25	1.95	5.39	a
Species richness (OTUs)							
	F. sylvatica	1	8	15	23	57	a
	P. abies	2	13	19	28	47	b
	P. sylvestris	6	15	21	25	45	b

The minimum, the 25 %, 50 % (median) and 75 % quantiles and maximum of the different parameters in samples from *F. sylvatica*, *P. abies* and *P. sylvestris* are given

[a] Different letters (a,b,c) indicate statistically significant differences between tree species for the corresponding parameter at $p < 0.05$

community structure, which was represented by both NMDS axes. The amount of water-soluble lignin fragments and the pH contributed to four models, and they were followed by the wood extractives and the water content, which were part of three different models. In models considering water-soluble lignin fragments or the water

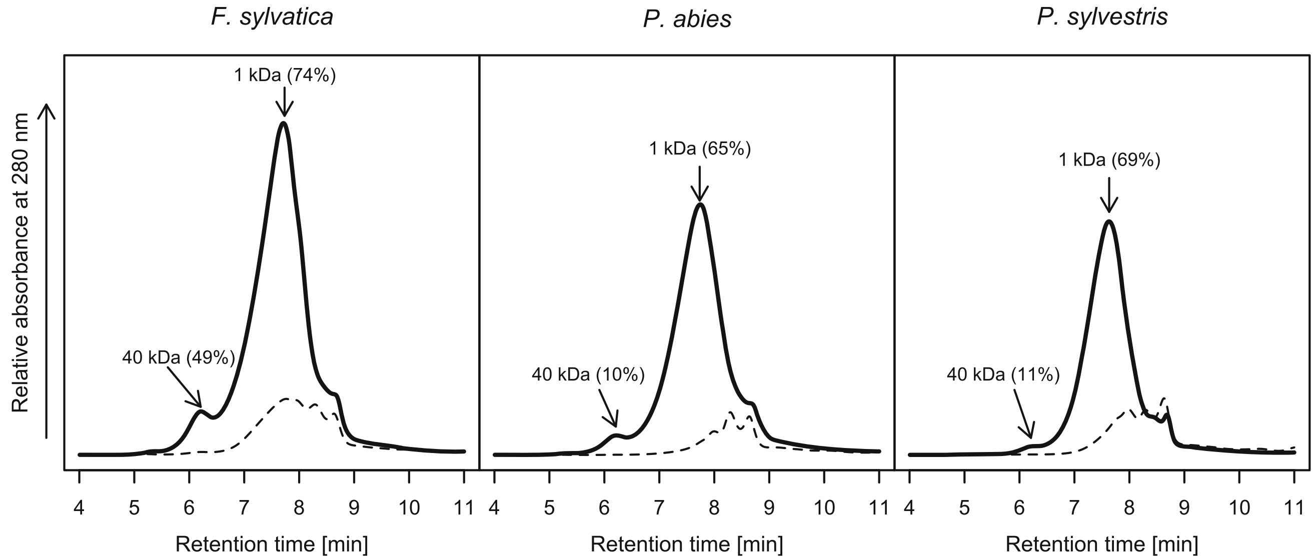

Fig. 1 Averaged chromatograms of water-soluble lignin fragments. The molecular mass distribution of lignin fragments in aqueous extracts of CWD samples (*solid line*) and sound wood (*dashed line*) of *F. sylvatica, P. abies* and *P. sylvestris* is shown. Corresponding molecular masses of the peaks are marked with *arrows,* and the percentages of samples exhibiting these peaks are given in *brackets*

Table 5 Selected final models for logistic models predicting the probability to detect activities of Lacc, GenP and MnP $\geq$1 mU g^{-1}

Variable	*F. sylvatica*			*P. abies*			*P.sylvestris*		
	Lacc	GenP	MnP	Lacc	GenP	MnP	Lacc	GenP	MnP
Intercept	2.70***	0.60***	0.56***	0.49**	−0.51**	−0.44*	0.22^{NS}	−0.44^{NS}	−0.97**
pH				0.93***		−0.36**^{,a}		−0.53$^{+,a}$	1.03**
Total lignin									
Extractives				−0.66**	−0.58**	−0.75***			
Water-soluble lignin fragments	0.81***			1.20***		0.82***			1.05**
Water content		0.47***			0.74***			0.75*	
Species richness	0.50*								
NMDS axis 1			0.30**				−0.88**		
NMDS axis 2				−1.01***	−0.45**	−0.59***			
Minor *r*-square	0.11	0.06	0.03	0.52	0.21	0.36	0.14	0.30	0.18

The estimated parameters and their significance of each variable, which is retained in the final model, are given together with the minor R^2 of the whole final model

Significance of the estimated parameters in the model is symbolized by: ^{NS} $p \geq 0.1$; $^{+}$ $0.05 \leq p < 0.1$; * $0.01 \leq p < 0.05$; ** $0.001 \leq p < 0.01$; *** $p < 0.001$

[a] Indicates that the estimated parameter is given for the quadratic term of this variable

content, the probability to detect the corresponding enzyme activities increased always together with them. In *P. abies* CWD, the probability to measure Lacc, GenP and MnP activities was negatively correlated with the content of extractives. In terms of pH, there was no general type of response. It depended on the type of enzyme and the tree species, whether the logistic regression was positively linear or quadratic. Species richness contributed only to the final model of Lacc in *F. sylvatica* CWD, and the total lignin content did not contribute to any model.

Correlations between enzyme activities, physico-chemical properties and species richness

To analyze which parameters promote the activities of Lacc, GenP and MnP, the correlation analyses were performed only for samples with enzyme activities $\geq$1 mU g^{-1}. All final models showed only low minor *r*-squares (Table 6). In particular, for *F. sylvatica* CWD, they were low, ranging from 0.09 to 0.11. For coniferous CWD, the models were better with a minor *r*-square between 0.15

Table 6 Selected final linear models of Lacc, GenP and MnP activities in dependence of physico-chemical parameters and fungal community parameters, modeled only with samples showing enzyme activity ≥1 mU g^{-1} of the corresponding enzyme

	F. sylvatica			*P. abies*			*P. sylvestris*		
	Lacc	GenP	MnP	Lacc	GenP	MnP	Lacc	GenP	MnP
Intercept	-0.2^{NS}	0.00^{NS}	-0.2^{NS}	0.00^{NS}	-0.09^{NS}	-0.01^{NS}	-0.04^{NS}	-0.02^{NS}	0.00^{NS}
pH		0.26***		−0.27**					
Total lignin						−0.45***			
Extractives						0.28*			
Water-soluble lignin fragments	0.26***		0.28***			0.23*			0.61**
Water content	0.15*		−0.23***	0.23*	0.41**			0.39*	
Species richness					−0.23*				
NMDS axis 1		−0.16**		−0.33***	−0.38**	−0.23*	−0.55***		
NMDS axis 2				−0.24**					
Minor *r*-square	0.11	0.09	0.09	0.27	0.19	0.39	0.34	0.15	0.38

The estimated parameters and their significance of each variable, which is retained in the final model, are given together with the minor R^2 of the whole final model

Significance of the estimated parameters in the model is symbolized by: ^{NS} $p \geq 0.1$; $^{+}$ $0.05 \leq p < 0.1$; * $0.01 \leq p < 0.05$; ** $0.001 \leq p < 0.01$; *** $p < 0.001$

and 0.38. The most frequent variables with five contributions to the final models were the water content and the fungal community represented by two NMDS axes. In relation to the water content, most correlations of the enzyme activities were positive with the exception of MnP activities in *F. sylvatica* CWD, which were negatively correlated. The amount of water-soluble lignin fragments was the second most important variable showing a positive effect on the activity of detectable enzymes. The pH was less influential with two contributions to the models, and the contents of total lignin and extractives as well as the species richness were only part of one final model each.

Discussion

Dead wood degradation by saprotrophic wood-inhabiting fungi is a complex process. They are metabolizing and mineralizing cellulose, hemicellulose and lignin to different extent and with different strategies. To make the polysaccharides better accessible for their hydrolytic enzymes like endoglucanase, cellobiohydrolase, *β*-glucosidase, xylanase and mannanase, they have to overcome the lignin barrier. For this purpose, white rot fungi apply a set of oxidoreductases (peroxidases and laccase) (Hatakka and Hammel 2011; Schwarze 2007).

Lacc, GenP and MnP activities are highly variable

Lacc, GenP and MnP activities detected in the analyzed CWD samples were often one or two orders of magnitude lower than enzyme activities typically found in "optimized" solid-state laboratory cultures of individual fungi (Fackler et al. 2006; Hahn et al. 2013; Liers et al. 2011). In general, there seems to be a wide variation in enzymatic activities in dead wood. Similar low enzyme activities as in the majority of our samples (Table 3) were measured by Valaskova et al. (2009) in strongly decayed wood within the range from 0 to 0.68 mU g^{-1} for Lacc and from 0.33 to 1.30 mU g^{-1} for MnP. In contrast, values up to 390 mU g^{-1} for Lacc and 1620 mU g^{-1} for MnP were found close to fruiting bodies of the WRF *Fomes fomentarius* in *F. sylvatica* logs (Větrovský et al. 2010), which is in the same order of magnitude as our observed maximum values. The large percentage of samples with relatively low Lacc, GenP and MnP activities (Tables 2, 3) may be a result of the high spatial variability of these enzymes. Each drill core across the logs covered altering and irregular zones of active lignocellulose degradation and zones with low or lacking enzyme activities (Boddy et al. 1989; Větrovský et al. 2010). As the result, local hot spots of enzymatic activities may have been overlooked. However, the high number of samples (701) in our study allows a realistic insight into the distribution of relevant oxidoreductase activities in CWD.

Lacc, GenP and MnP activities in *F. sylvatica* (broadleaved tree) were found to be significantly higher than in the two coniferous species (Table 3). This may be caused by different fungal communities, since different fungi prefer different tree species with their different characteristics (Schwarze 2007). For example, the here-studied tree species differed significantly in extractives, total lignin and wood density (Table 4). The low activities of the enzymes in coniferous wood suggest that it is rather degraded by

BRF than by WRF, which is in accordance with the fact that BRF preferably colonize coniferous wood (Ryvarden and Gilbertson 1993) and do not secrete peroxidases and scarcely Lacc (Hatakka and Hammel 2011). This assumption is further supported by the high number of coniferous wood samples (~25 %) with total lignin contents higher than 40 % compared to the low number of respective samples of *F. sylvatica* (~6 %). Nevertheless, the low but distinct Lacc, GenP and MnP activities found show that WRF do also occur in coniferous wood as it was reported, for example, for *P. abies* CWD (Rajala et al. 2012). The high percentage of small water-soluble lignin fragments detected in *P. abies* and *P. sylvestris* samples, which are characteristic for WRF attack (Liers et al. 2011), substantiates this fact since BRF do not cause the release of lignin fragments (Hahn et al., 2013). The high overall activities of oxidative enzymes observed in *F. sylvatica* samples imply vice versa that the decay of this tree species is dominated by WRF.

The first and second axis of the NMDS ordination for the fungal community is frequently part of the final model, which indicates the distinct influence of differences in the fungal community on the enzymatic activities and the probability to detect Lacc, GenP and MnP activities as incipiently hypothesized (H2). This is in accordance with different degradation rates previously observed for different fungal species compositions (Fukami et al. 2010) in *Nothofagus solandri* wood disks and the report of fungal community composition as the best predictor of mass loss in older stumps of *Q. robur* (van der Wal et al. 2015). In contrast, species richness appears only significant in one linear and one logistic model (Tables 5, 6), which clearly indicates that species richness is a good proxy neither for the fungal community nor for related processes.

Although the fungal community is without doubt responsible for the detected oxidative enzyme activities, their contribution to the final models is not such strong as it might be expected and the explanatory power of the final models is rather weak (Tables 5, 6). On the one hand, this finding might be a result of the complexity of individual communities per sample, which cannot be sufficiently accessed by statistics due to the stochastic fungal colonization process (Dix and Webster 1994) and the multidimensional development of fungal communities with diverse optional pathways (Boddy 2001; Stokland et al. 2012). Recently, van der Wal et al. (2015) demonstrated that the fungal community in old *Q. robur* stumps is similar to a randomly assembled community. Moreover, the status of development of each fungus and the status of interaction between the fungi will be relevant, too, since: (1) each fungus accomplishes an individual set and temporal pattern of extracellular oxidoreductase activities (Liers et al. 2011), (2) these activities are higher when a fungus is capturing fresh unoccupied wood (Hahn et al. 2013; Větrovský et al. 2010), (3) during interaction of several fungal species, activities of Lacc or MnP can increase (Baldrian 2004; Hiscox et al. 2010; Šnajdr et al. 2011), and (4) competition between fungi can hamper wood degradation (Fukasawa et al. 2009b). This complex interplay of fungal species may be the reason for the observed extreme variability (Table 3) and cannot be explained by solely analyzing the fungal community. For a better understanding of the secretion of fungal laccase and peroxidases, the analysis of the functional fungal community by expression studies for each enzyme based on transcriptomics will be useful (Kellner et al. 2014).

Last but not least, the low explanatory power may also be an effect of timescales. Lacc, GenP and MnP activities describe the current decay process (activity "snapshot"), in a time frame of days (or weeks at best) depending on enzyme stability, while all other physico-chemical properties describe the results of the whole past decay process. This applies also to the fungal community data used here, which cover all species from the past decay process, since the OTUs are derived from total DNA that includes DNA of living but also of dead and/or inactive fungi (Purahong and Krüger 2012).

Lacc, GenP and MnP activities are crucial for lignin conversion in CWD

The measured oxidative activities (Lacc, GenP, MnP) are representative for the most important extracellular enzymes involved in the chemical modification and degradation of lignin (Hatakka and Hammel 2011). The considerable number of samples where we detected relevant activities (Table 2) indicates their general significance for CWD decay. As a result of delignification, we found, in the majority of samples, distinct patterns of water-soluble lignin fragments (Fig. 1), similar to those detectable in solid-state pure cultures in the laboratory (Liers et al. 2011). But vice versa these fragments can also influence the production/activity of enzymes (feedback regulation). Notably, we found water-soluble lignin fragments to be one of the most important variables to predict the activities of Lacc and MnP as well as the probability to detect them (Tables 5, 6). There were always positive correlations with enzyme activities and the probability to detect them. This indicates the strong relation of Lacc and MnP to the process of lignin degradation. Contrary to our initial assumption, with total lignin, there was no logistic regression for all measured enzyme activities and only one linear model, namely that for MnP activity in *P. abies* CWD. This may be an effect of different timescales, in which total lignin is the result of the whole past decay process, while water-soluble lignin fragments are rather the result of short-time activities and hence are more related to the actual decay process similar to the activity of enzymes. Despite the strong relations between water-soluble lignin fragments and activities of Lacc as well as MnP, it is, for the time being, impossible to disentangle whether the enzymatic activities or the water-soluble lignin fragments are cause or effect.

According to the activities detected, Lacc was the most frequent enzyme in CWD (Table 2), which may be due to its involvement in different processes including fruiting body development, pigmentation of fungal spores, rhizomorph formation, detoxification reactions and sexual differentiation additional to lignin modification (Boddy 2000; Leonowicz et al. 2001). The fact that water-soluble lignin fragments were frequently part of the final models for Lacc in CWD (Tables 5, 6) is an indication for the contribution of Lacc to lignin modification, inasmuch as it facilitates the detoxification of phenolic wood ingredients and phenolic fragments formed by peroxidases, which on their part can affect fungal growth and peroxidase performance (Bollag et al. 1988; Leonowicz et al. 2001; Upadhyay and Hofrichter 1993). As a typical result of Lacc action, we found water-soluble lignin fragments with a predominant mass around 40 kDa, which is also characteristic for fungi secreting Lacc as sole extracellular oxidoreductase (e.g., *Xylaria* spp.; Liers et al. 2011). On the other hand, when looking at the molecular mass distribution as a whole, the 40-kDa fraction contributes only to a minor extent to the total amount of soluble lignin fragments (Fig. 1), which rather points to a limited role of Lacc in the overall degradation process.

The frequency of MnP and GenP activities in the samples was similar (Table 2); however, only in the final models for MnP, water-soluble lignin fragments were predictors and total lignin was it in the final model of MnP activity in *P. abies*. These relations to water-soluble lignin fragments and total lignin confirm the crucial role of MnP in lignin degradation, which was proposed after laboratory studies demonstrating the ability of MnP to depolymerize lignin and even to mineralize it to CO_2 (Hofrichter et al. 1999; Kapich et al. 1999). In this context, it is also noteworthy to mention that MnP is the most frequent type of peroxidase secreted not only by wood-rot WRF but also by leaf-litter dwelling basidiomycetes (Hofrichter et al. 2010; Lundell et al. 2014). The significance of MnP for ligninolysis was further emphasized by the frequency of a characteristic fraction of water-soluble lignin fragments (with a molecular mass around 1 kDa indicative for MnP attack) occurring in 70 % of all samples (Fig. 1; Hofrichter et al. 2001). Even though enzymatic MnP activities were not always detectable, the presence of the enzyme can be inferred from these fragments that are far more stable than the peroxidase protein itself (Liers et al. 2011).

Although in the final model for GenP activities in CWD, water-soluble lignin fragments did not appear, this does not necessarily mean that the peroxidases behind these activities are not involved in the degradation of lignin. It must be considered that the GenP activities measured here represent sum activities that rely on several fungal peroxidases (Kellner et al. 2014), among which only LiP and VP are truly ligninolytic (Hofrichter et al. 2010; Ruiz-Dueñas and Martínez 2009). Consequently, the relation of LiP and VP to water-soluble lignin fragments could be masked/corrupted by activities of GP, UPO and DyP (Hofrichter et al. 2010; Liers et al. 2011). In future studies, the use of more specific peroxidase substrates (that will have to be selected carefully) in combination with proteomic approaches will help to distinguish the different enzymes hiding behind GenP activities.

Optimum parameters for Lacc, GenP and MnP activities

In this study, the pH and the presence of metals (copper, iron, manganese) were found to be important boundary conditions for the extracellular oxidoreductase activities and their magnitude was in the range of suitable conditions determined in numerous laboratory tests (Baldrian 2006; Brown et al. 1990; Hofrichter et al. 2010; Kirk and Cullen 1998; Levin et al. 2005).

In some cases, the pH was an important predictor for the enzyme activities and the probability to detect them in the final models (Tables 5, 6). Most samples showed an acidic pH (Table 4), which is similar to the acidic pH optima (3.0–5.5) of purified Lacc, LiP, VP or MnP (Baldrian 2006; Hofrichter et al. 2010; Kirk and Cullen 1998), which supports the hypothesis that an acidic pH facilitates extracellular oxidoreductase activities related to lignin modification. Most contributions of pH to the final models (Tables 5, 6) were found for coniferous CWD. This could be the result of a stronger acidification by BRF that prefer this type of wood (Ryvarden and Gilbertson 1993) and secrete oxalic acid over their whole life cycle in contrast to WRF (Dutton et al. 1993; Shimada et al. 1997). Since the observed interquartile range of pH was quite small (Table 4), it is hard to interpret the different increasing, decreasing or quadratic functions of pH and the corresponding enzyme activity. This finding merely underlines the importance of pH for the functioning of extracellular oxidoreductases.

For essential heavy metals (Mn, Fe, Cu), it is not possible to disentangle their individual influence on the Lacc, GenP and MnP activities in CWD, since they were strongly correlated with each other and with water-soluble lignin fragments ($R = 0.48$–0.77; Online Resource: Table S4-S6). However, the univariate consideration before fitting the multivariate models revealed some positive effects of these metals on Lacc, GenP and MnP activities (Online Resource: Table S9 & S10), as hypothesized in the beginning (H1). It is well known from laboratory studies that minimal levels of these metals are indispensable for the synthesis and functioning of extracellular oxidoreductases (Baldrian 2006; Brown et al. 1990; Hofrichter et al. 2010; Levin et al. 2005).

The majority of manganese concentrations detected in the aqueous phase of wood samples (Online Resource: Table S8) were in the range (or higher) of the K_m values (Michaelis–Menten constants) of MnP for Mn^{2+}. Collected K_m data from the literature were used to calculate a mean value of 26 µM Mn^{2+} within a range from 4 to 124 µM ($n = 26$) (Online Resource: Table S11). Interestingly, between 25 and 75 % of the samples contained bioavailable manganese in concentrations that are usually used to assay MnP activities (100–500 µM) (Camarero et al. 1996; Fackler et al. 2006; Hofrichter et al. 1999). This strongly indicates that the natural concentration of bioavailable manganese in wood is sufficient to guarantee the functioning of MnP (that uses Mn^{3+} as actual oxidant) and thereby enables incipient lignin oxidation (Hofrichter 2002). The concentrations of copper in the aqueous phase of wood samples were five to six orders of magnitude lower than concentrations known to be toxic to fungi (Sanglimsuwan et al. 1993) and two orders of magnitude lower than the concentrations used to induce Lacc production in liquid laboratory cultures (Levin et al. 2005; Tavares et al. 2005). But it is noteworthy that there are remarkable differences between fungal species in the way they tolerate and respond to variant concentrations of copper ions (Baldrian 2003; Mäkelä et al. 2013). Furthermore, also the activity of MnP can be enhanced in the presence of copper as observed for *Trametes trogii* (Levin et al. 2005). Nevertheless, the copper concentrations (interquartile range 3–7 µM; Online Resource: Table S8)—and also the iron levels (interquartile range 13–53 µM; Online Resource: Table S8)—in wood were found to be sufficient for fungal growth and functioning of their enzymes. Copper and iron regulate the cellular metabolism and gene expression, and are also needed as cofactors in multiple core metabolic enzymes, receptors and trafficking of structural proteins (Baldrian 2003; Huffman and O'Halloran 2001; Lundell et al. 2014; Philpott 2006).

Water content is an important determinant of extracellular oxidoreductase activities

Density and water content of dead wood is permanently changing with the ongoing decay process. Thus, wood density decreases, and as a result, the water content increases, indicated by their strong negative correlation. The univariate consideration before fitting the multivariate models revealed that there are more significant univariate correlations and logistic regression with the tested enzymes for the water content than for wood density. The effect of density may be superposed by that of the water content. Wood density slowly decreases during the decay process, and in contrast, the water content is subject to fluctuations caused by precipitation or drought, which in turn will lead to responses by the fungi (Chambers et al. 2001). Different fungal species can also shape the water content during degradation to different extent (Boddy et al. 1989; Fukasawa et al. 2005). Therefore, only the water content was added to the maximal model for multivariate modeling.

The model selection demonstrated that the water content—as expected—is an important determining factor for activities of Lacc, GenP and MnP as well as for the probability to detect them in CWD (Tables 5, 6). This fits well to observations made for respiration rates as proxy for the fungal degradation activity, which is partly the result of lignin mineralization (Hagemann et al. 2010; Herrmann and Bauhus 2013; Hofrichter et al. 1999). In most cases, where the water content was retained in the final model, the activities of the corresponding enzymes and the probability to detect them increased with the water content. Only the activity of MnP in *F. sylvatica* CWD decreased with increasing water content. This may indicate that MnP is most important in the beginning of the decay process in *F. sylvatica* CWD, as the water content increased with ongoing decay. However, for respiration rates, both directions of response have been reported (Boddy 1983; Liu et al. 2006; Progar et al. 2000).

Conclusions

By analyzing 701 CDW samples of *F. sylvatica*, *P. abies* and *P. sylvestris* across three regions in Germany, we frequently found activities of extracellular oxidoreductases, i.e., laccase (Lacc), manganese peroxidase (MnP) and several other peroxidases [subsumed under general peroxidase (GenP) and also referred to as manganese-independent peroxidases (Morais et al. 2002)], which highlights their importance for the chemical modification and decomposition of lignin and hence for carbon cycling in forests. In particular, manganese-oxidizing peroxidases dominate the disintegration of lignin, which is supported by the observed patterns of water-soluble lignin fragments formed during wood decay and by the correlation and logistic regressions of water-soluble lignin fragments and MnP.

Lacc, GenP and MnP show complex and highly variable patterns in their activities, which are controlled by an array of factors including tree species, water content, water-soluble lignin fragments, pH and availability of manganese, copper and iron. As initially hypothesized, the fungal community structure was the most important determinant that influences the presence and activities of these enzymes. But low explanatory power of all final models indicates that the community structure and the important physico-chemical variables are not sufficient to explain the complexity of extracellular oxidoreductase patterns in CWD. The individual development of each fungus, the fungal identity and abundance as well as the status of interaction between the fungi may be more important. For a better explanation of fungal laccase and peroxidase occurrence in the future, functional analysis of the fungal community by RNA-based expression studies for individual enzyme will be necessary along with enzymatic and molecular analyses of (hemi)cellulytic hydrolases.

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Compliance with ethical standards

This material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. Hereby I confirm that the submission of the manuscript has been approved by all of the authors and the International Institute Zittau (TU Dresden) as institution where the majority of work was carried out. I certify on behalf of all coauthors that there is neither a financial nor a non-financial conflict of interest.

Conflict of interest The authors declare that they have no conflict of interest.

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